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The hypoxic kidney: pathogenesis and noncoding RNA-based therapeutic strategies

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Abstract: Acute kidney injury (AKI) is a disease entity of major importance, affecting approximately 6% of all patients on the intensive care unit. The mortality rate exceeds 60%. AKI is related to several underlying conditions, including sepsis, nephrotoxicity or major surgery. Ischaemia reperfusion injury or hypoxic conditions may lead to severe injury of the kidney and is associated with a steep decline in survival rates of patients. At present, AKI is diagnosed on the basis of creatinine levels and urine output. Novel markers and knowledge of their pathophysiological role is of major importance for targeted therapeutic interventions. Noncoding RNAs (ncRNAs) have recently been introduced and are the subject of intensive research initiatives. They are arbitrarily separated into small ncRNAs (1e;200 nucleotides) and long ncRNAs (lncRNAs, ge;200 nucleotides). Whereas small ncRNAs such as microRNAs have been extensively studied over the past several years, investigations into the role of linear lncRNAs and circular RNAs (circRNAs) are largely lacking. The present review article therefore aims to elucidate in detail the role of microRNAs, lncRNAs and circRNAs in animal models as well as patients with ischaemic AKI and to describe their use as biomarkers as well as their potential use as therapeutics.

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The hypoxic kidney: pathogenesis and noncoding RNA-based therapeutic strategies

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Summary

Acute kidney injury (AKI) is a disease entity of major importance, affecting approximately 6% of all patients on the intensive care unit. The mortality rate exceeds 60%. AKI is related to several underlying conditions, including sepsis, nephrotoxicity or major surgery. Ischaemia reperfusion injury or hypoxic conditions may lead to severe injury of the kidney and is associated with a steep decline in survival rates of patients. At present, AKI is diagnosed on the basis of creatinine levels and urine output. Novel markers and knowledge of their pathophysiological role is of major importance for targeted therapeutic interventions. Noncoding RNAs (ncRNAs) have recently been introduced and are the subject of intensive research initiatives. They are arbitrarily separated into small ncRNAs (≤ 200 nucleotides) and long ncRNAs (lncRNAs, ≥ 200 nucleotides). Whereas small ncRNAs such as microRNAs have been extensively studied over the past several years, investigations into the role of linear lncRNAs and circular RNAs (circRNAs) are largely lacking. The present review article therefore aims to elucidate in detail the role of microRNAs, lncRNAs and circRNAs in animal models as well as patients with ischaemic AKI and to describe their use as biomarkers as well as their potential use as therapeutics.

Keywords: acute kidney injury, long non-coding RNA, microRNAs, circular RNAs, mortality, renal replacement therapy

Introduction

Acute kidney injury (AKI) is associated with considerable morbidity and was identified previously as an independent risk factor for mortality of patients [1]. Even though significant advances have been made over the past several years regarding best supportive care, AKI on the intensive care unit is still highly prevalent and associated with a high mortality [2]. As evidenced by a major multinational, multicentre study involving more than 30,000 critically-ill patients with AKI on the intensive care unit, the in-hospital mortality exceeds 60% [3]. Thirteen percent of survivors who are subsequently discharged from hospital progress to end-stage kidney disease and remain on dialysis [3]. Ischaemia/reperfusion injury (I/R injury) of the kidney represents one of the major risk factors for the development

of AKI [4]. A variety of different injurious insults in native kidneys (e.g., during cardiac surgery) culminate in I/R injury [4]. In addition, it is also an unavoidable phenomenon in transplanted kidneys owing to the transplantation procedure itself [5]. Because of its prevalence, it represents a major socioeconomic health problem [4]. During ischaemic AKI, a transient drop in blood flow to the kidney is followed by a reperfusion period. Reperfusion itself, though vital to restoration of kidney function, is associated with significant additional cellular injury [6]. Among a multitude of different signalling pathways, non-coding RNAs may also significantly contribute to the induction and resolution of renal I/R injury.

RNA transcripts without protein-coding potential represent more than 90% of the human genome [7, 8]. These non-coding RNA transcripts (ncRNAs) are arbitrarily separated into long ncRNAs (lncRNAs, ≥ 200 nucleotides) and small ncRNAs (≤ 200 nucleotides). The biogenesis and function of small ncRNAs such as microRNAs (miRNAs) has previously been described in detail [6–8]. In contrast, knowledge of the role and function of lncRNAs is largely lacking. The lncRNA class comprises both linear lncRNAs (named by default as lncRNAs) and circular RNAs (circRNAs). Current knowledge of the role of these three categories of ncRNA molecules (miRNAs, lncRNAs, circRNAs) in kidney function and ischaemic AKI is reviewed in the following paragraphs.

Pathophysiology of renal ischaemia/reperfusion injury

As mentioned in the introduction, during ischaemic AKI a transient drop in blood flow to the kidney is followed by a reperfusion period, which is associated with significant additional cellular injury [6]. The damage inflicted by tissue ischaemia is subsequently aggravated by a dramatic surge in reactive oxygen and nitrogen species during reperfusion [9, 10]. These induce protein modifications, lipid oxidation and DNA double strand breaks, finally culminating in endothelial dysfunction, neutrophil adherence to endothelium and transendothelial migration, the release of inflammatory mediators, cellular calcium overload and eventually cell death [9, 10]. In the kidney, blood flow to the outer medulla is disproportionately reduced compared with the reduction in total blood flow [9, 10]. Thus, epithelial cell

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injury is mainly detected in the S3 segment of the proximal tubule, located in the outer medulla [9, 10]. Interplay of several events contributes to the cellular injury observed in the kidney. The damaged endothelium interacts with and activates inflammatory cells through enhanced expression of adhesion molecules (e.g., intracellular adhesion molecule-1 [ICAM-1], selectins) [9, 10]. This interaction in turn contributes to obstruction of capillaries and postcapillary venules, further activation and transmigration of leucocytes, production of cytokines and inflammation in tubular epithelial cells [9, 10]. Capillary rarefaction in the inner stripe of the outer medulla ensues as a result of the development of chronic hypoxia and is an important contributor to post-AKI tubulointerstitial fibrosis and progression to chronic kidney disease [9, 10]. Cell polarity and cytoskeletal arrangement is severely impaired in proximal tubular epithelial cells during ischaemia [9, 10]. Important phenotypical changes are loss of the proximal tubule brush border as well as loss of polarity and derangement of adhesion molecules and other membrane proteins and disruption of cell-cell interactions at adherent and tight junctions [9, 10].

MicroRNA biogenesis and function

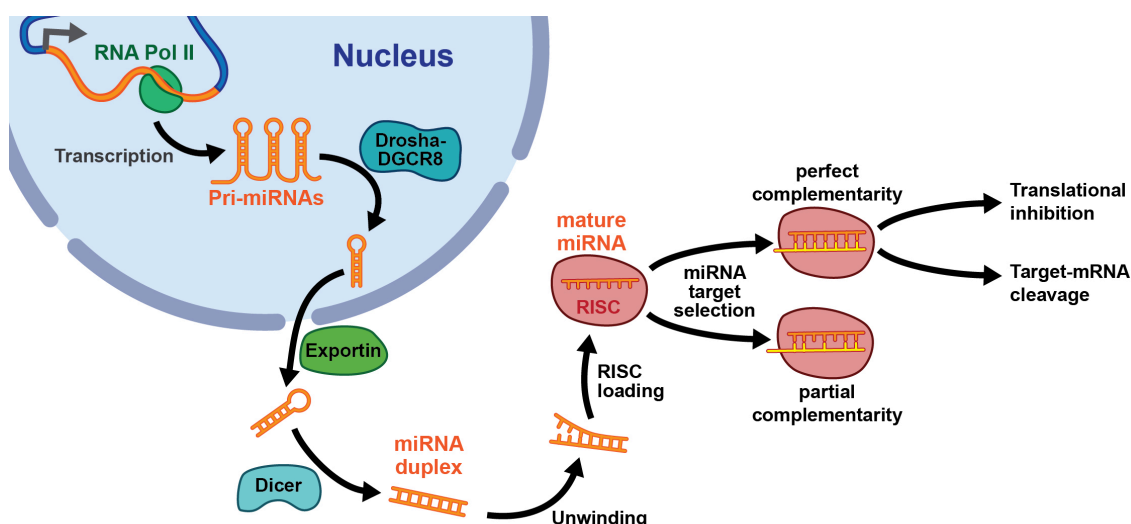
MicroRNAs (miRNAs) have been extensively studied over the past 10 years and found to have a major role in ischaemic AKI [11]. MiRNAs are small noncoding RNA transcripts with a length of approximately 22 nucleotides, which may be perceived as being responsible for fine tuning of protein expression by targeting the 3'-untranslated region of mRNA transcripts, thereby inducing transcriptional repression or transcript degradation [11]. The first miRNA, lin-4, was identified in *Caenorhabditis elegans* during investigation of genetic loci responsible for temporal control of postembryonic development [12, 13]. The number of identified mature miRNAs in humans exceeds 2000 (www.mirbase.org; release 21), thereby representing 1% of all human genes [14]. MiRNA biogenesis can be viewed as a two-step process (see [fig. 1](#)). Transcription of miRNA genes by RNA polymerase II results in primary miRNA transcripts, which are subsequently processed in

the nucleus by the ribonuclease Drosha into precursor miRNAs with a length of 70 nucleotides [11]. Thereafter, a Ran-GTP-dependent nucleocytoplasmic cargo transporter named exportin 5 transports precursor miRNAs into the cytosol [11]. Here, they are further processed by a second ribonuclease, called Dicer, into a small, double-stranded RNA duplex, which is composed of an miRNA guide strand and its complementary passenger strand (miRNA*) [11]. In order to exert its function, the miRNA guide strand (in most cases) is incorporated into the RNA-induced silencing complex (RISC), where, with argonaute and other proteins, the 3'-untranslated region of mRNAs is targeted, thereby leading to repression of protein translation or degradation of mRNA [11]. In most cases, miRNA* is degraded, but rarely it can also function as a mature miRNA [11]. MiRNA function can be modulated by miRNA antagonists, so-called anti-miRs (chemically engineered oligonucleotides targeting specific miRNAs) and miRNA mimics [11]. These miRNA modulators may have therapeutic potential in the treatment of patients with kidney disease, including AKI [11]. In addition to their intracellular regulation and role in the diagnosis and treatment of various diseases, they are also secreted into blood and urine and may therefore serve as biomarkers of disease and response to therapy [15].

MiRNAs in animal models of ischaemic acute kidney injury

In 2010, an initial study was published that investigated the role of miRNAs in AKI by modulation of the miRNA biogenesis machinery through specific deletion of Dicer in renal proximal tubular epithelium in mice. It was shown that after bilateral I/R injury of the kidney, Dicer knockout mice displayed preserved kidney function and reduced tissue damage compared with littermate controls [16]. MiR-192 was associated with AKI in rats [17]. We previously showed miR-24 to be enriched in the kidney following I/R injury in mice and humans [18]. MiR-24 was specifically enriched in renal endothelial and tubular epithelial cells after I/R induction. Through regulation of its targets H2A histone family, member X, the sphingo-

Figure 1: Biogenesis and function of microRNAs (see text). RISC = RNA-induced silencing complex



sine-1-phosphate receptor 1, and haem oxygenase 1, miR-24 induced apoptosis of these cells. Silencing of miR-24 in mice following I/R injury resulted in a significant improvement of survival and kidney function, a reduction of apoptosis, improved histological tubular epithelial injury and less infiltration of inflammatory cells.

In unilateral renal I/R injury in mice, nine miRNAs were shown to be regulated differentially compared with control animals (miR-21, miR-20a, miR-146a, miR-199a-3p, miR-214, miR-192, miR-187, miR-805, and miR-194) [10, 19]. This signature was confirmed in immunodeficient recombination activating gene-2 (RAG-2) / common γ -chain double-knockout mice, suggesting that the miRNA expression is independent of influx of inflammatory cells [10, 19]. MiR-21 expression increased in proliferating tubular epithelial cells, and silencing of miR-21 promoted apoptosis [10, 20]. MiR-127 was demonstrated to be altered in a rat model of renal I/R injury. Here, miR-127 was postulated to be transcriptionally activated by hypoxia-inducible factor-1 α (HIF-1 α) and shown to target kinesin family member 3B (KIF3B) [10, 21]. Endothelial progenitor cells were shown to secrete miRNA-enriched microvesicles, which ameliorated murine renal I/R injury [10, 21]. Pro-angiogenic and anti-apoptotic miR-126 and miR-296 were found to be included in microvesicles. Injection of microvesicles derived from endothelial progenitor cells into rats subjected to renal I/R injury led to proliferation of tubular cells and a reduction in the number of apoptotic tubular cells, and decreased the infiltration of leucocytes [10, 22]. MiR-21 was found to contribute to renoprotection in a process termed ischaemic preconditioning [10, 23]. MiR-21 was associated with a significant amelioration, while antimir-21-mediated silencing promoted renal I/R injury [10, 23]. These effects were mediated by modulation of the miR-21 target programmed cell death protein 4 (PDCD4). MiR-21 was transcriptionally activated by HIF-1 α [10, 23]. MiR-182-5p and miR-21-3p were associated with the development of AKI in kidney transplant patients [24]. *In vivo* silencing of miR-182-5p in a model of AKI in rats preserved renal function [25]. MiR-126 has previously been shown to have a beneficial effect regarding the development of AKI in mice [26]. Haematopoietic overexpression of miR-126 increased neovascularisation, preserved kidney function and increased numbers of bone marrow-derived endothelial cells. The numbers of circulating Lin⁻/Sca-1⁺/cKit⁺ haematopoietic stem and progenitor cells were increased [26].

MiRNAs as biomarkers of acute kidney injury

Expression levels of miR-709, miR-217 and miR-696 have been shown to be altered in plasma and kidney tissue following bilateral renal I/R injury [27]. MiR-10a was elevated in plasma 1 hour after renal I/R injury in rats and thereby outperformed levels of creatinine (6 hours) and urea (12 hours) in detection of renal injury [28]. It was also previously shown to be altered in rat kidney following deep hypothermic circulatory arrest [29]. MiR-21 and -320 are increased in plasma and reduced in urine of rats following AKI [30]. The expression of miR-200a increases in the plasma and kidneys of mice following AKI [31]. We were able to show that miRNAs, specifically miR-210, detected in the plasma of critically ill patients with AKI serve

as powerful and independent predictors of mortality in this patient cohort [32]. Urinary miRs were shown to be specifically decreased during acute T-cell mediated renal allograft rejection and to correlate with kidney function at 1 year after transplantation [33].

Relevant microRNAs in animals and human models of AKI are summarised in [table 1](#).

Long noncoding RNAs – biogenesis and postulated function

The majority of the human genome is transcribed into RNA transcripts with little or no protein coding potential. In fact, more than 98% are so called noncoding RNAs (ncRNAs), which are arbitrarily separated into long ncRNAs (lncRNAs, ≥ 200 nucleotides) and small ncRNAs (≤ 200 nucleotides) [7]. Information regarding the function of lncRNAs, as opposed to miRNAs, is largely lacking. However, it is now widely accepted that lncRNAs have major roles in epigenetic processes and cell function in the developmental phase, as well as during disease [34]. LncRNAs are novel regulatory RNA species, which may function as master regulators modifying the expression of mRNAs and miRNAs, and altering chromatin architecture. LncRNAs may either be nuclear-enriched or primarily found in the cytosol. Nuclear lncRNAs may associate with the chromatin architecture of genes on the same chromosome (in cis) or on another chromosome (in trans) [34]. Cytoplasmic lncRNAs may function as competing endogenous RNAs and thereby regulate the function of miRNAs [34]. A functional annotation has not been forwarded yet. This has led to the categorisation of lncRNAs on the basis of their location in the genome in relation to protein-coding genes. LncRNAs may therefore be viewed as: sense, antisense, bidirectional promoter-associated, intronic, intergenic and enhancer-associated [34]. In view of the lack of a functional annotation, Howard Chang and co-workers have proposed a mechanistic model regarding the role of lncRNAs [34, 35]. According to this suggested classification, lncRNAs are classified as: (1) signal lncRNAs, which are spatiotemporally transcribed in response to developmental cues, cellular context or diverse stimuli; (2) decoy lncRNAs, which impact on transcriptional regulation by titrating away transcription factors and other proteins from chromatin; (3) guide lncRNAs, which sequester ribonucleoprotein complexes and direct them to chromatin and other specific targets; and (4) scaffold lncRNAs, which interact with multiple partners to form a chromatin modifying complex (see [fig. 2](#)) [34, 35]. To date, the roles of a small number of lncRNAs have been characterised in more detail. The long intergenic RNA (lincRNA) Air has been shown to regulate imprinting, whereas X-chromosome inactivation has been shown to be influenced by Xist (X-inactive specific transcript) [34]. The lncRNA H19 has a tumour-suppressive effect and is imprinted with maternal expression [34].

LncRNAs in acute kidney injury

Recently, the hypoxic and inflammatory dysregulation pattern of lncRNAs in cultured human proximal tubular epithelial cells was investigated [36]. The proximal tubular

epithelial cells were exposed to hypoxia and treated with a cytokine cocktail implicated in clinical AKI (interleukin-6 [IL-6], tumour necrosis factor- α [TNF- α], and interferon- γ). A total number of 667 annotated lncRNAs were found, but only 14 different lncRNAs overlapped between treatment conditions. Hypoxia-sensitive lncRNAs were located in the vicinity of protein-coding genes, including those with roles in the Wnt/ β -catenin signalling pathway, ATP metabolism and vitamin D receptor activation, as well as cellular differentiation. MIR210HG, linc-ATP13A4-8 and linc-KIAA1737-2 were amongst the most highly upregulated transcripts. These were detectable in human kidney tissue as well as microdissected proximal tubules from kidney transplant recipients. Addition of blood samples of sepsis patients to cultured cells induced expression of all investigated lncRNAs.

The lncRNA RANTES (regulated on activation, normal T-cell expressed and secreted) is induced in mice with I/R injury [37]. Kidney dysfunction, renal inflammation and tissue injury in RANTES knockout mice were shown to be ameliorated following renal I/R injury. Nuclear factor- κ B (NF- κ B) was transcriptionally activated RANTES. Moreover, a HIF-1 α -regulated lncRNA, termed PRINS (psoriasis susceptibility-related RNA gene induced by stress), was proposed to interact with RANTES under hypoxic conditions. In a mouse model of unilateral ureteral obstruction, a large number of dysregulated lncRNAs was recently identified by means of RNA sequencing [38]. The antisense lncRNA Arid2-IR was found to be differentially regulated and associated with progressive renal inflammation in a unilateral ureteral obstruction model involving Smad3-knockout and wild-type littermate control animals [39]. Recently, our group analysed the contribution of the

hypoxia-regulated lncRNA metastasis associated lung adenocarcinoma transcript 1 (Malat1) to ischaemic AKI [40]. Malat1 was highly induced in kidney biopsies and plasma of patients with AKI as well as in a mouse model of renal I/R injury. In addition, its expression increased in *ex vivo* sorted hypoxic endothelial and proximal tubular epithelial cells, as well as in cultured endothelial and proximal tubular epithelial cells. HIF-1 α functioned as a transcriptional activator of Malat1. Malat1 silencing impaired the proliferation rate and reduced the number of ECs in the S-phase of the cell cycle. *In vivo*, Malat1 knockout and wild-type mice did not exhibit differences in the levels of renal injury, capillary rarefaction and fibrosis as well as survival rate and kidney function. RNA sequencing did not reveal differences in the expression of small RNAs and mRNAs between groups. LncRNA activated by transforming growth factor- β (TGF- β) (lncRNA-ATB) is highly upregulated in kidney biopsies of patients with acute renal allograft rejection [41].

LncRNAs as biomarkers of acute kidney injury

Our group previously reported an initial study regarding the release and biomarker potential of lncRNAs in patients with AKI. Here, the intronic antisense lncRNA transcript predicting survival in AKI (TapsAKI, alternative nomenclature: MGAT3AS1) was shown to be massively increased in blood and kidney biopsies of patients with AKI [42]. TapsAKI proved to be an independent and powerful predictor of 28-day survival [42]. TapsAKI may be released from endothelial and tubular epithelial cells under stress conditions, since its expression level was increased by hypoxia or chemical anoxia (ATP depletion).

Table 1: MicroRNAs, their targets and effects in renal ischaemia/reperfusion injury.

MicroRNA	Target	Organ/cell type	Pathophysiological effects	Organism	Reference
miR-132, miR-362 and miR-379	/	Kidney / proximal tubular cell	Dicer deletion	Mouse	[16]
miR-21, miR-20a, miR-146a, miR-199a-3p, miR-214, miR-192, miR-187, miR-805 and miR-194	/	Kidney	Signature of kidney ischaemia/reperfusion injury	Mouse	[10, 19]
miR-127	Kinesin family member 3B	Kidney / proximal tubular cell	Changes in cell adhesion and cytoskeleton structure	Rat	[21]
miR-126 and miR-296	/	EPC-derived MVs / proximal tubular cell	Inhibition of capillary rarefaction, glomerulosclerosis, and tubulointerstitial fibrosis	Mouse	[22]
miR-21	Programmed cell death protein 4	Kidney / proximal tubular cell	Reduction of renal injury	Mouse	[23]
miR-192	/	plasma	Biomarker of AKI	Rat	[17]
miR-24	H2A.X, sphingosine 1-phosphate receptor 1, haem oxygenase-1	Proximal tubular cell, endothelial cell	Amelioration of AKI	Mouse	[18]
miR-182-5p	/	/	Improvement of kidney function and morphology after AKI	Rat	[25]
miR-126	/	Haematopoietic overexpression of miR-126	increased neovascularisation, preserved kidney function and increased numbers of bone marrow-derived endothelial cells	Mouse	[26]
miR-709, miR-217 and miR-696	/	Plasma	Biomarker of AKI	Mouse	[27]
miR-10a	/	Plasma	Biomarker of AKI	Rat	[28]
miR-21 and miR-320	/	Plasma	Biomarker of AKI	Rat	[30]
miR-200a	/	Plasma	Biomarker of AKI	Mouse	[31]
miR-210	/	Plasma	Biomarker of AKI	Human	[32]
miR-210	/	Urine	Biomarker of acute T cell-mediated rejection of renal allografts	Human	[33]

AKI = acute kidney injury; EPC = endothelial progenitor cells; H2A.X = H2A histone family member X; MV = microvesicles

We subsequently investigated kidney biopsies and urine of kidney transplant patients with acute T cell-mediated renal allograft rejection. Three intergenic lncRNAs were identified: LNC-MYH13-3:1, RP11-395P13.3-001 and RP11-354P17.15-001 [43]. RP11-354P17.15-001 predicted acute rejection and loss of kidney function at 1 year after transplantation. Exposure of cultured tubular epithelial cells to the inflammatory cytokine IL-6, increased the levels of all lncRNAs, but only RP11-395P13.3-001 and RP11-354P17.15-001 expression increased in the cell culture supernatant, indicating that these lncRNAs might be secreted under inflammatory conditions.

LncRNAs with roles in rodent and human models of AKI are summarised in [table 2](#).

Circular RNAs – biogenesis and postulated function

Circular RNAs (circRNAs) are suggested to be part of a so-called competing endogenous RNA class and may function as miRNA sponge transcripts. They are ubiquitously distributed and have diverse functions. CircRNAs are formed as single-stranded, circular molecules, in which the

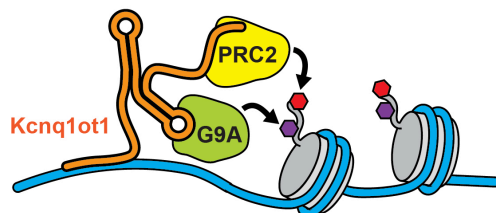
3' and 5' ends are covalently linked [44–46]. The majority of circRNAs are generated by a process termed back-splicing. Here, a splice donor is joined with an upstream splice acceptor [45, 46]. They are ~100 nucleotides in length and are highly present in the eukaryotic transcriptome and abundant in exosomes [47]. “Exonic” circRNAs (formed from exons) can be differentiated from “intronic” circRNAs, which contain a 2'-5' carbon linkage at the branch point stemming from introns. It is currently believed that circRNAs are not translated into protein, since they are lacking a 5' cap [48]. A characteristic feature of circRNAs is the “head-to-tail” splice junction, where exons are organised in reverse order compared with their chromosomal localisation. This is a consequence of the backsplicing reaction.

The RNA binding proteins Muscleblind [45], Quaking [49] or RNA binding motif protein 20 [50] may contribute to circRNA biogenesis. On the other hand, the RNA-editing enzyme adenosine deaminase RNA specific (ADAR1) blocks circRNAs biogenesis [51].

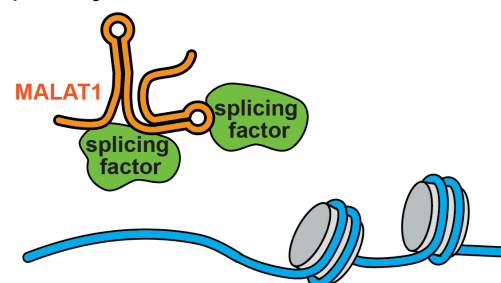
Platelets, neutrophils, B cells and haematopoietic stem cells may be important sources of circRNAs [52–54]. In addition, circRNAs may be actively secreted and detected in exosomes or small vesicles [55].

Figure 2: Mechanistic classification of lncRNAs as signal lncRNAs, which are spatiotemporally transcribed (a), decoy lncRNAs, which impact on transcriptional regulation by titrating away transcription factors (b), guide lncRNAs, which sequester ribonucleoprotein complexes and direct them to chromatin and other specific targets (c) and scaffold lncRNAs, which interact with multiple partners to form a chromatin modifying complex (d). Kcnq1ot1 = potassium voltage-gated channel subfamily Q member 1 antisense transcript 1; MALAT1 = metastasis-associated lung adenocarcinoma transcript 1; FENDRR = Fox F1 adjacent non-coding developmental regulatory RNA; PRC1 = polycomb repressive complex 1; PRC2 = polycomb repressive complex 2

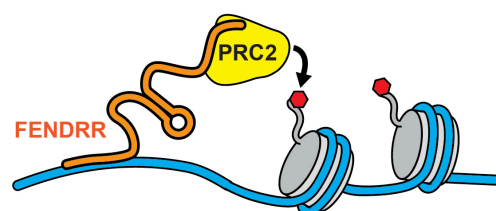
a) Signal lncRNA



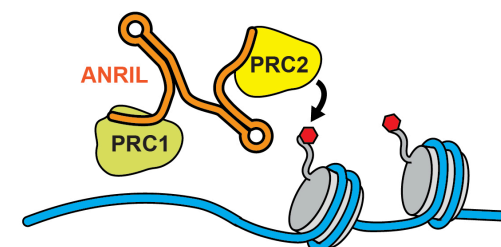
b) Decoy lncRNA



c) Guide lncRNA



d) Scaffold lncRNA



 Histone 3 lysine 9 methylation
 Histone 3 lysine 27 methylation

 lncRNA
 Histone

Table 2: LncRNAs, their targets and pathophysiological effects in acute kidney injury.

LncRNA	Target	Annotation	Pathophysiological effects	Organism	Reference
MIR210HG	Unknown	Intergenic	Induced by hypoxia and cytokine treatment in cultured proximal tubular epithelial cells	Human	[36]
linc-ATP13A4-8	Unknown	Intergenic	Induced by hypoxia and cytokine treatment in cultured proximal tubular epithelial cells	Human	[36]
linc-KIAA1737-2	Unknown	Intergenic	Induced by hypoxia and cytokine treatment in cultured proximal tubular epithelial cells	Human	[36]
PRINS	HIF-1 α	Intronic	induced in hypoxia, regulated in RANTES knockout mice	Mouse	[37]
Arid2-IR	NF- κ B, IL-1 β	Intronic	Inflammation and fibrosis	Mouse	[39]
MALAT1	Unknown	Intergenic	ischaemia/reperfusion-injury with lack of effect	Mouse	[40]
TapsAKI	Unknown	Antisense	Biomarker (plasma) in acute kidney injury	Human	[42]
RP11-354P17.15-001	Unknown	Intergenic	Biomarker (urine) of acute T cell-mediated rejection of renal allografts	Human	[43]

Arid2-IR = AT-rich interactive domain-containing protein 2 intronic region; HIF-1 α = ; IL-1 β = interleukin-1 β ; MALAT1 = metastasis associated lung adenocarcinoma transcript 1; NF- κ B = nuclear factor- κ B; PRINS = psoriasis susceptibility-related RNA gene induced by stress; RANTES = regulated on activation, normal T cell expressed and secreted; TapsAKI = transcript predicting survival in AKI

Recently, it was shown that circRNAs may function as remarkably stable biomarkers in human blood as a result of their resistance to exonucleases through circularisation [56]. Several studies have highlighted their biomarker potential in patients with atherosclerosis [57], disorders of the central nervous system [58], degenerative diseases [45], and cancers [55, 59].

Circular RNAs in ischaemic acute kidney injury

The literature on the role of circRNAs in the kidney and especially AKI is scarce. An initial study showed that circRNAs may be important for renal development [60]. RNA sequencing confirmed that circRNAs are present and enriched in the kidney [61]. Several circRNAs have been shown to be altered in murine ischaemic AKI [62]. Our group has recently shown that the novel circRNA ciRS-126 predicts survival of critically ill patients with AKI [63]. We performed a global circRNA expression analysis using RNA isolated from blood of patients with AKI. This expression analysis yielded a number of dysregulated circRNAs. Circulating concentrations of three novel circRNAs, identified with array, were validated by means of quantitative polymerase chain-reaction tests (qPCR) in blood of patients with AKI. Circular RNA sponge of miR-126 (or ciRs-126) was the most altered compared with controls. CiRs-126 was shown to bioinformatically sponge miR-126-5p, which was found to be highly suppressed in AKI patients and hypoxic endothelial cells. Cox regression and Kaplan-Meier curve analysis revealed ciRs-126 to be an independent predictor of 28-day survival. Circulating concentrations of circRNAs, and more specifically ciRs-126, in patients with AKI may act as a predictor of mortality in this patient cohort.

Conclusions

Pharmacological treatment of patients with AKI is limited. A targeted and specific therapy in proximal tubular cells of the S3 segment or endothelial cells of the outer medulla, which are the primary target cells in ischaemic AKI, to halt or even reverse the progression of AKI development is largely lacking. Noncoding RNAs may be therapeutically used to ameliorate or retard disease progression, as recently shown by us regarding miR-21 silencing in murine di-

abetic nephropathy [64] and chronic allograft dysfunction following kidney transplantation [65], as well as miR-24 [66] inhibition regarding murine renal ischaemia reperfusion injury. MicroRNAs are highly conserved between different species and therefore hold huge potential as biomarkers and therapeutic targets in humans with kidney disease. LncRNAs are novel regulatory RNA species, which have been shown to critically impact on various cellular functions and may therefore be an ideal candidate for therapeutic interventions. In general, lncRNA conservation between species is low, thereby limiting their applicability for therapeutic intervention in humans following initial validation in animal studies. However, their functional and structural (secondary structure) homology might be much higher across species. Another obstacle is the fact that certain lncRNAs have several transcripts. Thus, the identification of specific mechanisms of action is not trivial.

The elucidation of the role of circRNAs is still in its infancy. It is currently unclear, how and to what extent they contribute to kidney disease. Future studies will aim to identify novel circRNAs with implications in kidney disease. Ultimately, modulation of noncoding RNAs may be a viable therapeutic option in the treatment of patients with kidney disease.

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